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NTP-CERHR Expert Panel Report on the Reproductive and Developmental Toxicity of Acrylamide

Comments submitted by:

Kerry L. Dearfield, Ph.D.
U.S. Environmental Protection Agency
Office of the Science Advisor (8105R)
1200 Pennsylvania Ave., NW
Washington, DC 20460
202-564-6486
dearfield.kerry@epa.gov

1. In addition to the reproductive and developmental toxicity posed by a chemical, I believe a major concern for the CERHR is the risk a chemical poses to the induced effects (primarily induced genetic disease) in the offspring of exposed parents ("heritable risk"). When the information is available and the methodologies to evaluate such a heritable risk are available, as for acrylamide, the evaluation of heritable risk is critical in the CERHR report. This acrylamide report details the appropriate germ cell studies, but its conclusion appears to fall short of a logical and robust characterization of potential germ cell risk.

2. "The Expert Panel expressed minimal concern for acrylamide-induced heritable effects in the general population" (p. 151). The Expert Panel further "recognizes that dose-response information for these effects is limited" (p. 151), primarily because of the lack of testing at low dose levels where reproductive and developmental toxic effects are observed (p. 145).

However, the Expert Panel concludes elsewhere (p. 145) "there are sufficient data to conclude that acrylamide induces transmissible genetic damage in male germ cells of mice in the form of reciprocal translocations and gene mutations. Such effects can lead to genetic disorders and infertility in subsequent generations." Further on: "...it is likely that such effects would occur at lower dose levels." I agree that such effects would occur at lower dose levels. However, having qualitatively stated such a conclusion on p. 145, it doesn't seem to connect with the final conclusion on p. 151 with the expression of minimal concern without quantification of risk. The conclusion on p.145 suggests very strongly that acrylamide is a potential human germ-cell mutagen and at least qualitatively suggests that a higher level of concern for heritable effects is warranted.

Unlike reproductive and developmental effects which assume a non-linear dose response, the germ cell data are assumed to follow a linear dose response relationship. You shouldn't use a LOAEL based on reproductive and developmental effects to characterize heritable risk since the LOAEL is not likely to be applicable to the characterization of heritable risk. For example, there

are germ cell effects (e.g., dominant lethal effects (postimplantation loss)) that are seen at similar dose levels as the reproductive and developmental LOAELs. There probably are increased heritable risks at doses below those at the reproductive and developmental LOAELs.

3. I am of the opinion that the issue of heritable risk posed by acrylamide and the heritable risk (germ cell mutagenicity) conclusion needs to be more fully fleshed out and quantified if the Expert Panel provides a judgment about the level of concern for acrylamide. For example, the EPA's Guidelines for Mutagenicity Risk Assessment ("Mutagenicity Guidelines") focus on heritable risk and detail a process for addressing germ cell risk assessment. For acrylamide, the risk assessment would begin with a qualitative discussion about the hazard acrylamide presents to germ cells and would discuss how the evidence is sufficient for acrylamide interaction in the mammalian gonad. The evidence as detailed in the Expert Panel report provides valid, and ample, support that acrylamide is a potential human germ-cell mutagen. It needs to be mentioned that we don't have actual data derived from a human germ cell study to say definitively that acrylamide <u>is</u> a human germ-cell mutagen. But the animal data and other data (including other human data, ADME data) provide the ample evidence that acrylamide is a potential human germ-cell mutagen.

4. The next step for the acrylamide heritable assessment as per the Mutagenicity Guidelines is to quantify the potential germ cell risk. This is outlined as a two step process in the Mutagenicity Guidelines. First, the heritable effect per unit of exposure (dose response) for acrylamide needs to be detailed. This is appropriately done using the data from the heritable translocation studies. The same could be done for the specific locus study, but the data are more complete for the heritable translocation studies and these studies provide reproducibility of the translocation effect as well. Also, the germ cell targets in the mode of action discussion are more consistent with the translocation data (there is probably some contribution from gene mutations a la the specific locus information; this probably needs discussed qualitatively and suggests that the estimates from the heritable translocation data may actually be underestimates of the total germ cell risk). An assumption of linearity is made for the dose response curve for the translocation data. This is the same assumption Drs. Hattis and Favor support and is generally supported by the data. The translocation data demonstrate a linear component and linearity is further supported by the dominant lethal dose response data.

The second step is to determine the relationship between the mutation rate found for acrylamide and disease incidence. The doubling dose approach (discussed in Dearfield et al., 1995) is determined by the experts in mutagenicity risk assessment as appropriate to determine the relationship between acrylamide mutation rate and disease incidence. There is greater uncertainty about the number of disease-associated loci in the other approach (modified direct) discussed in Dearfield et al. in contrast to the greater certainty about the spontaneous rate (for translocations) in humans in the doubling dose approach. Finally, to characterize the quantitative risk in terms of the estimated increase in genetic disease per generation (one of the measures the Mutagenicity Guidelines details for reporting), as expressed per one million offspring, calculations found in Dearfield et al. (1995) are appropriate to apply here.

Below are the appropriate equation and values to use in the equation. I provide a table of the calculated potential germ cell risks expressed as the estimated increase in genetic disease per one million offspring. This table can be inserted into an appropriate section of the report. For exposures, I used representative exposures found in the human exposures summary (pp. 19-20). For the doubling dose, I used two values from the heritable translocation data representing the highest and lowest values found so that a plausible range of germ cell risk can be provided. I rounded the numbers to "whole" numbers (i.e., number of offspring).

Equation (detailed in Dearfield et al., 1995):

new disease in offspring = REF X $\text{Spon}_{\text{human}}$ X (D/DD) X N

REF	=	risk extrapolation factor (between rodent (mice) and humans) (ICPEMC, 1993); value is 0.2
$\text{Spon}_{\text{human}}$	=	Overall spontaneous rate (translocations) to dominant disease alleles; value is 1.9 per 1000 newborns (i.e., 0.0019) (Lyon et al., 1983)
D	=	Dose or human exposure (this is from the exposure assessment)
DD	=	doubling dose estimated in the mouse (dose that doubles the spontaneous mutation rate in the mouse); obtained from the dose response data; lowest value from the data = 0.39 mg/kg, highest value from the data = 25 mg/kg
N	=	number of offspring descendent from exposed parent(s); value = one million, so the number of new genetic diseases is expressed per million offspring

From the table (below), for example, with a DD of 0.39 mg/kg, exposure for dermal exposure (assuming 25% absorption) would estimate up to 1 offspring with induced genetic disease per million offspring from exposed parents(s). Likewise, exposure for food ingestion for the general population would estimate up to 4 offspring with induced genetic disease per ten million offspring from exposed parent(s). For 3 kg babies exposed to acrylamide via mother's milk, this exposure would estimate a range of up to 5 offspring per 100 million exposed individuals to 3 offspring per million exposed individuals with induced genetic disease.

Table of estimated values

Endpoint	Mouse dose (mg/kg) (dose schedule)	Doubling Dose (DD) (mg/kg)	Number of induced genetic diseases per million offspring					
			Dermal	Food ingestion (general)	Food ingestion (baby - mother's milk)	Water ingestion	Inhala-tion (mean)	Inhala-tion (upper bound)
			0.0011 (mg/kg bw/day)	0.00043 (mg/kg bw/day)	0.0033 (mg/kg bw/day)	0.000014 (mg/kg bw/day)	0.0014 (mg/kg bw/day)	0.043 (mg/kg bw/day)
Chromosomal alterations	250 [a] (5 X 50)	0.39	1	0.4	3	0.01	1	40
Chromosomal alterations	Combined [a,b] (50 as single combined w/ 250 (5 X 50))	25	0.02	0.007	0.05	0.0002	0.02	0.7

See Dearfield et al., 1995 for details on mouse dose and schedule (data from [a] Adler (1990) and combined from [a] Adler (1990) and [b]Adler et al.(1994))

5. The estimates given above are derived from the translocation data as the endpoint examined. However, there are several caveats that need to be presented in the report discussion and conclusion that indicates that this only presents a portion of the possible heritable risk from acrylamide. These estimates are derived from only one genetic endpoint, translocations. However, we know from the specific locus data, there are also gene mutations being induced in offspring and these can potentially lead to an increase in genetic disease. The translocation data present estimates for *dominant* diseases; however, we know there is risk, potentially more, due to the induction of *recessive* mutations that won't be expressed in the first generation. This aspect is a very important risk factor to future generations that really isn't quantitated here. This needs mentioned.

A major component of the potential risk to acrylamide are the germ cell targets and the timing of the exposure relative to those targets (particularly the time frame when affected sperm are utilized). For example, if exposures are sporadic, then affected sperm (from the post-meiotic germ cell targets affected) may be cleared before reproduction and the risk is lessened. However, if exposure is continuous, there would be a continual risk for affected offspring. If the germ cell target is spermatogonia (as suggested by the specific locus data), then the timing of future exposures isn't as relevant as the risk would remain from the original exposure to the spermatogonia.

All of these caveats suggest that the estimates just from the translocation data may actually be underestimates for the overall heritable risk. We don't know today how to combine the estimates from the translocation and specific locus data as well as combine the estimates for dominant and recessive mutation-related diseases.

6. I agree with Drs. Hattis and Favor that a "higher level of concern" than "minimal concern" for heritable risk be applied to acrylamide. This chemical is a prototypical germ cell mutagen and the exposure to humans is documented - it is a potential human germ-cell mutagen and presents a heritable risk (in the form of potential increased genetic disease) to future generations. This conclusion needs to be more explicitly stated - this seems to be well within the purview of CERHR. In the past, we usually haven't had such data to make this type of conclusion. We do for acrylamide and so this would be a sorely missed opportunity to address the potential heritable risk concern by CERHR. Hopefully I provide enough material to flesh out the heritable risk characterization.

Based on the quantitation presented above, the Expert Panel may rethink its concern level. For the general population, the heritable risk numbers appear low. But for occupational exposures, for example, the risk increases (also see Dearfield et al., 1995). However, with the caveats mentioned above, these risk estimates for offspring from all exposed persons are most likely underestimates. With the Expert Panel conclusion that it is likely that such risks would occur at lower dose levels and now with the estimates quantified (as potential underestimates) for comparison, a case can be made for a higher concern level than minimal for acrylamide heritable risk.

7. To emphasize my point that to make a judgment of minimal concern without quantitation doesn't make sense (especially with the large amount of evidence qualitatively to the contrary), I present a final thought. The Expert Panel states a conclusion that heritable effects would occur at lower dose levels, but does not quantify. The reason for not quantifying is questionable, i.e., absence of low dose-response data. Yet we can extrapolate to these lower doses much like we do for cancer risk estimates at lower doses, particularly with linear dose responses. We have the data for tumors from the 2 year bioassays at "higher" levels, and we have the models for extrapolating to lower doses, so we calculate estimates at the lower doses. Similarly, we have the dose response data for acrylamide germ cell effects at the "higher" animal tested doses, we have the models to estimate induced disease at lower doses (like for most cancers, linearity is assumed), and we have a process about how we can quantify acrylamide heritable risk. Like for cancer risk, we may say at actual exposure levels we might not have a concern, but we still make the qualitative argument that there is greater than a minimal concern for cancer in general. I believe this is a parallel case for acrylamide heritable risk that needs to made in the CERHR report.

8. References I think are not already in the Expert Panel Report:

ICPEMC (International Commission for Protection Against Environmental Mutagens and Carcinogens) (1993) Use of in vivo genetic toxicology data to construct human risk assessments, Final report submitted to Department of Health, Canada, July 1993, Contract No. 3138.

Lyon, M., I.-D. Adler, B. Bridges, L. Ehrenberg, L. Golberg, K. Kilian, S. Kondo, E. Moustacchi, A. Putrament, K. Sankaranarayanan, F. Sobels, R. Sram, G. Streisinger, and K. Sundaram (1983) International Commission for Protection Against Environmental Mutagens and Carcinogens (ICPEMC), Committee 4 Final Report, Estimation of genetic risks and increased incidence of genetic disease due to environmental mutagens, Mutation Research 115: 255-291.

U.S. Environmental Protection Agency (1986) Guidelines for Mutagenicity Risk Assessment, Federal Register 51: 340006-34012.

9. I can be available to discuss this with the Expert Panel should they be interested.